Application of hybrid imaging techniques in immunological and neoplastic diseases

Ph.D. Thesis

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1. Introduction

1.1. Hybrid imaging

The development of medical imaging and radiopharmacology was founded on the discoveries of two scientists at the end of the 19th century. Wilhelm Conrad Roentgen discovered X-ray in 1895, which was a milestone as it provided the first insight into the internal structure of the body. In 1896, Henri Becquerel demonstrated that certain natural substances emit radiation and that their presence can be detected. Marie Curie and Pierre Curie confirmed that uranium and polonium also have this property, and they named this phenomenon radioactivity. György Hevesy developed the tracer principle, which essentially involves introducing such a small amount of radioactive material into the human body that it does not interfere with molecular processes, but its distribution can be detected, thus providing accurate information about the body's metabolic functions. His discovery, for which he received the Nobel Prize in 1943, offers excellent opportunities in diagnostics and therapy. Hybrid nuclear medicine imaging is playing an increasingly important role in diagnostics, as it allows different imaging methods to be combined, thereby improving diagnostic accuracy. Currently, the most widely used hybrid imaging technologies include positron emission tomography (PET) and computed tomography (CT) or a combination of single photon emission computed tomography (SPECT) and CT systems. The advantage of hybrid imaging is that it allows the simultaneous combination of information obtained from functional and morphological imaging.

1.2. The role of hybrid imaging in clinical practice

1.2.1. The role of [^{18}F]FDG-PET/CT in inflammatory conditions

2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) is a glucose analogue that enters cells via mechanisms similar to those of glucose uptake. In inflamed tissues, an increased rate of glycolysis - occurring to a greater extent than in normal cells - forms the pathophysiological basis of FDG PET imaging in infectious and inflammatory conditions. Large-vessel vasculitis is a granulomatous inflammation of the vessel wall of unknown etiology that may involve the aorta and its major branches. The clinical presentation of large-vessel vasculitis is characterized by elevated, yet disease-nonspecific, inflammatory parameters - erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) - together with often atypical and difficult-to-classify symptoms corresponding to the anatomical localization of the affected large arteries. The limited specificity of these clinical and laboratory features increases diagnostic uncertainty and frequently delays early recognition of the disease. The diagnostic performance of [^{18}F]FDG PET in the detection of large-vessel vasculitis is characterized by high sensitivity (87-90%) and

also by a high specificity (73-98%). Vascular wall activity can be assessed both visually and by quantitative methods.

1.2.2. Preoperative Planning in Liver Surgery

The incidence of liver tumors is increasing worldwide, including Hungary. Advances in modern surgical techniques and imaging modalities have enabled the broader application of liver resections in both benign and malignant conditions. Despite the progress in liver surgery, posthepatectomy liver failure (PHLF) remains one of the most feared complications following liver resection, with an incidence ranging from 9% to 30% after extended resections. One of the main determinants of postoperative liver failure is insufficient residual hepatocyte function, most commonly due to an inadequately sized and functionally insufficient future liver remnant (FLR).

Mebrofenin (N-(3-bromo-2,4,6-trimethylphenyl carbamoyl-methyl)-iminodiacetic acid) is a lipophilic hepatobiliary tracer that, when labeled with ^{99m}Tc, forms a radiopharmaceutical well suited for hepatobiliary scintigraphy. As a lidocaine derivative, mebrofenin is taken up by hepatocytes via organic anion transporting polypeptides (OATP1B1 and OATP1B3) and is subsequently excreted into the bile through multidrug resistance-associated protein 2 (MRP2). Global and segmental liver function can be characterized using quantitative parameters, and additional SPECT/CT acquisitions enable functional volumetry. Hepatobiliary scintigraphy is playing an increasingly important role in preoperative risk assessment prior to major liver resections. This is particularly relevant in cases with heterogeneous functional distribution, such as after liver parenchyma-modulating procedures, as well as in patients with underlying liver cirrhosis.

1.2.3. Neuroendocrine Tumors

Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) are typically slow-growing, well-differentiated neoplasms. Despite their indolent behavior, they may develop distant metastases even at a small primary tumor size and at an early stage of the disease, most commonly to the liver. Neuroendocrine tumors are characterized by the overexpression of somatostatin receptors (SSTRs), predominantly the subtype 2 and 5 receptors, which enables the use of targeted, receptor-based diagnostic imaging and therapeutic approaches. In Hungary, somatostatin receptor scintigraphy is commonly performed using [^{99m}Tc]Tc-EDDA/HYNIC-TOC or [^{111}In-DTPA-D-Phe^{1}]octreotide radiopharmaceuticals, in combination with SPECT/CT. Technetium-based imaging demonstrates sensitivity and specificity in the range of

80–90% and, owing to its lower radiation burden, wider availability, and improved cost-effectiveness, may represent a preferable option in routine clinical practice.

Peptide receptor radionuclide therapy (PRRT) involves the administration of a somatostatin analogue labeled with a beta-emitting radionuclide, most commonly [^{177}Lu]Lu-DOTA-TATE. This compound binds with high affinity to somatostatin receptors expressed on the surface of tumor cells, thereby enabling targeted therapy. The beta radiation emitted during radioactive decay induces local DNA damage, selectively destroying tumor cells.

2. Aims

1. To clinically evaluate the role of [^{18}F]FDG PET/CT in patients with suspected large-vessel vasculitis and in the assessment of therapeutic response during remission.

2. To evaluate the clinical role of dynamic [^{99m}Tc]Tc-mebrofenin SPECT/CT-derived functional parameters in liver surgery.

A. To compare clinical laboratory-based scoring systems used to characterize global liver function with the SPECT/CT-based hepatic clearance value.

B. To investigate the predictive value of the indocyanine green (ICG) elimination test, two-dimensional ultrasound shear wave elastography, and dynamic [^{99m}Tc]Tc-mebrofenin SPECT/CT-derived parameters in the prediction of postoperative liver failure.

C. To assess the effectiveness of liver parenchyma-modulating procedures in improving liver function by comparing pre- and post-interventional measurements.

3. To demonstrate the role of different hybrid imaging modalities ([^{99m}Tc]Tc-EDDA/HYNIC-TOC and [^{99m}Tc]Tc-mebrofenin SPECT/CT) and peptide receptor radionuclide therapy (PRRT) in the diagnosis, treatment planning, and follow-up of a patient with a rare clinical course of pancreatic neuroendocrine tumor.

3. Materials and methods

3.1. [^{18}F]FDG-PET/CT in large vessel vasculitis

3.1.1. Patients

Between December 2015 and January 2019, a total of 43 patients were prospectively enrolled from the Department of Rheumatology and Immunology, University of Szeged. Due to suspected primary large-vessel vasculitis, [^{18}F]FDG PET/CT was performed in 30 patients. The study population was divided into two groups: the first group consisted of patients (n = 17/30) who had not received glucocorticoid therapy at the time of imaging (steroid-naive group), whereas the second group (n = 13/30) included patients who were already receiving steroid treatment at the time of PET/CT due to manifest clinical symptoms. In an additional 11 cases, [^{18}F]FDG PET/CT was performed to confirm disease relapse in patients with known large-vessel vasculitis, while in 2 cases the examination was conducted to verify disease remission. Following the [^{18}F]FDG PET/CT examination, patients were clinically followed for a minimum of 6 months. The control group consisted of 10 oncological patients in whom inflammatory or autoimmune disease could be reasonably excluded and whose malignancy did not involve mediastinal, pulmonary, or hepatic structures.

3.1.2. Imaging

[^{18}F]FDG PET/CT examinations were performed in accordance with the guidelines of the European Association of Nuclear Medicine (EANM) for infectious and inflammatory diseases. Patients received a mean administered activity of 355 MBq (range: 230–593 MBq) of [^{18}F]FDG. Image acquisition was initiated 60 minutes after tracer injection. [^{18}F]FDG PET/CT imaging was performed using a GE Discovery ST 4 PET/CT scanner (GE Healthcare, Amersham, United Kingdom).

3.1.3. Visual assessment

Visual assessment of [^{18}F]FDG uptake in the large vessels was performed using the scoring system proposed by Meller and colleagues: 0 - no increased [^{18}F]FDG uptake; 1 - [^{18}F]FDG uptake lower than physiological hepatic uptake; 2 - uptake equal to hepatic activity; and 3 - uptake higher than hepatic activity. The [^{18}F]FDG PET examination was considered positive for active large-vessel vasculitis when radiotracer uptake in the large vessels was equal to or exceeded physiological liver activity (visual score 2 or 3) and demonstrated a diffuse uptake pattern.

3.1.4. Quantitative assessment

For quantitative analysis, the arterial wall was manually delineated in each patient within four vascular segments: (1) the supra-aortic branches, (2) the thoracic aorta, (3) the abdominal aorta, and (4) the common iliac arteries. Regions of interest (ROIs) were drawn on axial CT slices, while sagittal and coronal images were used to verify accurate ROI placement and proper anatomical alignment. Following image fusion, the manually defined ROIs were applied to the corresponding PET slices. For quantitative evaluation of arterial wall [^{18}F]FDG uptake, maximum standardized uptake values (SUVmax) within each ROI were measured. The liver was selected as the reference region, and SUVmax values of both the vascular segments and the liver were determined, as well as their ratios.

3.2. Methods of preoperative assessment before extended liver resection

3.2.1. Patients and inclusion criteria

In this prospective study, a total of 35 patients were enrolled between January 2022 and August 2023 from the Department of Surgery, University of Szeged. The inclusion criteria were as follows: planned major liver resection, clinically confirmed compensated liver function, and the absence of contraindications related to general anesthesia.

3.2.2. Preoperative assessment of liver function

Prior to liver resection, the MELD-Na (Model for End-Stage Liver Disease including serum sodium), APRI (aspartate aminotransferase-to-platelet ratio index), and CTP (Child-Turcotte-Pugh) scores were calculated, and the indocyanine green (ICG) elimination test was evaluated. The laboratory parameters required for these assessments, the ICG measurement, and the dynamic [^{99m}Tc]Tc-mebrofenin SPECT/CT examination were all performed within the same week. On the day of the dynamic [^{99m}Tc]Tc-mebrofenin SPECT/CT examination, elastography measurements were obtained using a LOGIQ E9 ultrasound system (GE HealthCare, United States) to assess the elasticity of the future liver remnant (FLR). These measurements were used to estimate the degree of parenchymal fibrosis.

3.2.3. Methodology of dynamic [^{99m}Tc]Tc-mebrofenin SPECT/CT

All 35 patients included in the study were required to fast for at least 4 hours prior to the examination. The radiopharmaceutical was prepared on the day of the investigation in accordance with the manufacturer's instructions (MEDI-RADIOPHARMA Ltd.) and labeled locally with the ^{99m}Tc radionuclide before administration. Image acquisition was performed using a triple-head integrated SPECT/CT system (Mediso AnyScan TRIO, Mediso Medical

Imaging Systems Ltd., Budapest, Hungary) equipped with low-energy high-resolution (LEHR), parallel-hole collimators. SPECT acquisition was carried out over a 360° orbit with 96 projections, an acquisition time of 6 seconds per projection, a 128 × 128 matrix, and a pixel size of 4.22 mm. The energy window was centered on the 140 keV photopeak of ^{99m}Tc with a 20% window width. Prior to SPECT acquisition, a native low-dose CT scan was obtained for anatomical localization and attenuation correction (120 kV, 70 mAs). Patients were positioned supine. In all cases, the FOV included the heart, liver, and biliary system down to the common bile duct (ductus choledochus). The radiopharmaceutical was administered intravenously into an upper limb, which was positioned outside the FOV to avoid interference.

Dynamic SPECT imaging was performed following intravenous administration of [^{99m}Tc]Tc-mebrofenin (300 MBq) in continuous acquisition mode according to the default Mediso protocol, with consecutive SPECT data collections at 192-second intervals. Time-activity curves (TACs) were generated from the continuous three-dimensional datasets based on accurately delineated volumes of interest (VOIs) defined on the SPECT images.

On the SPECT datasets, the following regions were manually delineated: the blood pool (BP), including the mediastinal great vessels and the heart; the liver; the future liver remnant (FLR); and the entire field of view (FOV). Total liver filtration (TL-F, %/min) and future liver remnant filtration (FLR-F, %/min) were calculated from the time-activity curves within the 150-350 second interval following tracer injection, using the Ekman formula. TL-F and FLR-F values were normalized to body surface area (BSA, %/min/m^2) to account for interindividual metabolic differences, with BSA calculated according to the Mosteller formula. From the early, purely parenchymal-phase SPECT acquisition-prior to the onset of biliary excretion - the functional volumetric contribution of the FLR (FLR-FV%) was calculated by dividing the total counts within the FLR VOI by the total hepatic counts and multiplying the result by 100 to express it as a percentage. No biliary activity was observed during this parenchymal phase. The volumetric proportion of the FLR (FLR-V%) was determined as the ratio of FLR volume (FLR-V) to the tumor-free total liver volume (TL-V), multiplied by 100 and expressed as a percentage.

Among the 35 enrolled patients, FLR rate was below the desired threshold in 13 cases based on the initial dynamic [^{99m}Tc]Tc-mebrofenin SPECT/CT examination. Consequently, parenchyma-modulating interventions were performed prior to surgery, followed by a control dynamic [^{99m}Tc]Tc-mebrofenin SPECT/CT examination using the same imaging protocol.

4. Results

4.1. Results of [^{18}F]FDG PET/CT-based assessment in large-vessel vasculitis

4.1.1. Visual assessment results

A total of 212 (53 x 4) vascular segments from 53 patients were evaluated both visually and quantitatively. Based on visual assessment, among the 43 patients examined for suspected large-vessel vasculitis, increased [^{18}F]FDG uptake higher than hepatic activity (visual score: 3) or uptake equal to hepatic activity (visual score: 2) was identified in 28 vascular segments in 10 patients and was therefore considered a positive finding. In these cases, the pattern of [^{18}F]FDG uptake was diffuse, and active large-vessel vasculitis was suspected based on the pathological tracer uptake; in five patients, three or more vascular regions were involved. Among the patients included in the study, primary large-vessel vasculitis was diagnosed in five cases, whereas disease relapse was identified in an additional five patients, findings that were subsequently confirmed by clinical follow-up. Among patients without [^{18}F]FDG PET/CT findings indicative of active large-vessel vasculitis, increased [^{18}F]FDG uptake consistent with other inflammatory conditions was observed in eight cases.

4.1.2. Quantitative assessment results

During quantitative analysis, liver-normalized SUVmax values of visually positive arterial wall segments in visually positive cases were compared with those of arterial segments from visually negative patients and from the control group. Statistical analysis using the Kruskal-Wallis test revealed a significant difference among the groups ($p = 6.92 \times 10^{-19}$).

In patients with visually active large-vessel vasculitis and positive [^{18}F]FDG PET findings, the liver-normalized SUVmax values of visually positive arterial wall segments (median: 1.21 ± 0.24) were significantly higher than those of arterial segments in visually negative patients (median: 0.76 ± 0.11; $p = 3.33 \times 10^{-16}$) as well as those observed in the control group (median: 0.67 ± 0.08; $p = 4.52 \times 10^{-12}$). Moreover, liver-normalized SUVmax values of arterial wall segments in visually negative patients were significantly higher than those of the control group ($p = 6.64 \times 10^{-15}$).

4.1.3. Effect of steroids in active large-vessel vasculitis

Within the visually [^{18}F]FDG-positive patient group, liver-normalized SUVmax values of the affected vascular segments were significantly higher in steroid-naive patients (3 patients, 11 vascular segments; mean SUVmax: 1.43 ± 0.29) compared with patients who were already

receiving steroid therapy at the time of imaging (7 patients, 17 vascular segments; mean liver-normalized SUVmax: 1.17 ± 0.11; p = 0.005).

4.1.4. Assessment of remission

In two [^{18}F]FDG-positive patients with large-vessel vasculitis, follow-up [^{18}F]FDG PET/CT examinations were performed after successful treatment. In parallel with the resolution of clinical symptoms, previously positive vascular segments demonstrated [^{18}F]FDG negativity.

4.1.5. Results of laboratory parameters

C-reactive protein (CRP) levels were significantly higher in [^{18}F]FDG PET/CT-positive patients compared with the visually negative group (42.56 ± 29.63 vs. 15.92 ± 27.50; p = 0.002). In contrast, no statistically significant difference was observed in erythrocyte sedimentation rate (ESR) values between the two groups (54.75 ± 35.23 vs. 36.05 ± 37.45; p = 0.07).

4.2. Clinical significance of [^{99m}Tc]Tc-mebrofenin SPECT/CT-based functional parameters

4.2.1. Correlation with global liver function parameters

Total liver filtration determined by dynamic [^{99m}Tc]Tc-mebrofenin SPECT/CT showed a significant negative correlation with the MELD-Na score (ρ = -0.42; p = 0.04) as well as with the ICG-R15 value (ρ = -0.48; p = 0.018).

4.2.2. Prediction of PHLF based on ROC analysis

Based on the results of ROC analysis, FLR filtration proved to be a significant predictor of clinically relevant posthepatectomy liver failure (PHLF grades B + C), demonstrating excellent discriminatory performance (AUC = 0.947; p = 0.006). The optimal cutoff value determined by the Youden index was: 2.72%/min/m². Several additional variables also showed promising performance, including FLR-FV% (AUC = 0.810) and FLR-V% (AUC = 0.800), however, based on the data of our study population, these results did not reach statistical significance (p = 0.052, and p = 0.063). The elasticity value measured within the FLR was not a significant predictor of clinically relevant PHLF.

4.2.3. Effect of parenchymal modulation procedures

Among the 35 participants enrolled in the prospective study, preoperative parenchyma-modulating procedures were performed in 13 non-cirrhotic patients. Prior to parenchyma modulation, the median total liver filtration was 4.99 %/min/m² (IQR: 4.37-6.14 %/min/m²). Following modulation, this value increased slightly to 5.21 %/min/m² (IQR: 4.76-6.19 %/min/m²). However, this increase was not consistent, as the interquartile ranges showed

substantial overlap, and the difference between the two measurements was not statistically significant ($p = 0.88$). Before parenchyma modulation, the median FLR-V% was 31.51% (IQR: 24.45-36.60%), which increased to 46.37% after the intervention (IQR: 41.01-54.63%). This pronounced increase was statistically significant ($p < 0.0001$). Similarly, the baseline median FLR-FV% was 29.23% (IQR: 21.62-34.23%) rising to 53.32% (IQR: 46.11-65.25%) after modulation, which also represented a statistically significant increase ($p < 0.0001$).

FLR filtration also increased significantly, with the median value rising from 1.42 %/min/m² (IQR: 0.99-1.77) to 2.79 %/min/m² (IQR: 2.30-3.90) following parenchyma modulation ($p < 0.0001$). Finally, after modulation, the median ultrasound-measured FLR stiffness increased from 1.37 m/s (IQR: 1.30-1.49 m/s) to 1.47 m/s (IQR: 1.37-1.58 m/s); however, this difference was not statistically significant ($p = 0.44$).

5. Case presentation

Pancreatic neuroendocrine tumors (PanNETs) are rare neoplasms with heterogeneous biological behavior, ranging from slowly progressive forms to aggressive disease courses. Advances in nuclear medicine have enabled the application of modern functional imaging techniques in both the diagnosis and treatment of these tumors. In a 45-year-old female patient, a grade 1 PanNET was diagnosed with multiple liver metastases. Planning of complex surgical interventions - including distal pancreatic resection, splenectomy, extended liver resection, and radiofrequency ablation - was supported by [^{99m}Tc]Tc-EDDA/HYNIC-TOC SPECT/CT and preoperative [^{99m}Tc]Tc-mebrofenin-based functional liver volumetry. Dynamic [^{99m}Tc]Tc-mebrofenin SPECT/CT enabled precise assessment of functional liver volume and objective evaluation of the effectiveness of parenchyma-modulating procedures, thereby contributing to the prevention of posthepatectomy liver failure. Two-dimensional shear wave elastography (2D-SWE) was applied for the noninvasive assessment of liver fibrosis. Somatostatin receptor scintigraphy also played a crucial role in disease follow-up. Recurrent disease was treated with somatostatin analog therapy (SSA), peptide receptor radionuclide therapy (PRRT) with [^{177}Lu]Lu-DOTA-TATE, and surgical resection. Despite tumor progression to grade 3 - reflected by an increase in the Ki-67 index from 1% to 30% - the patient remained in good general condition with an ECOG performance status of 0 at 53 months after diagnosis.

This case highlights the pivotal role of functional imaging in therapeutic decision-making throughout the entire disease course, underscores the clinical significance of modern nuclear medicine, and illustrates the dynamic nature of PanNETs.

6. Conclusions

1. [^{18}F]FDG-PET/CT can be effectively applied to assess disease activity and extent in patients with large-vessel vasculitis who have not yet received glucocorticoid therapy.

2. Glucocorticoid treatment reduces active vascular wall [^{18}F]FDG uptake, thereby increasing the risk of false-negative findings; consequently, the timing of PET/CT imaging is of critical importance.

3. [^{18}F]FDG-PET/CT is suitable for confirming therapeutic response in patients with large-vessel vasculitis in remission.

4. To our knowledge, this study demonstrates a previously unreported correlation between global liver filtration and clinically accepted scoring systems used to assess total liver function, as well as with the indocyanine green (ICG) test, thereby supporting the reliability of this dynamic SPECT-derived parameter.

5. In addition, to the best of our knowledge, this study is the first to identify a functional parameter - FLR filtration - derived from dynamic [^{99m}Tc]Tc-mebrofenin SPECT imaging that is capable of predicting the development of clinically relevant posthepatectomy liver failure (PHLF grades B + C).

6. We demonstrated the impact of parenchyma-modulating procedures using parameters derived from dynamic [^{99m}Tc]Tc-mebrofenin SPECT/CT, which effectively support the optimization of preoperative strategy.

7. Through a case of a neuroendocrine tumor with multiple liver metastases, we demonstrated the importance of preoperative dynamic [^{99m}Tc]Tc-mebrofenin SPECT/CT in guiding a change in surgical strategy.

8. We demonstrated the role of [^{99m}Tc]Tc-EDDA/HYNIC-TOC imaging and peptide receptor radionuclide therapy (PRRT) in mapping disease extent and in determining individualized treatment in pancreatic neuroendocrine tumors.

7. List of publications

MTMT number: 10029211

Full papers directly related to the thesis

1. **Bakos A**, Libor L, Vasas B, et al. New Horizons: The Evolution of Nuclear Medicine in the Diagnosis and Treatment of Pancreatic Neuroendocrine Tumors-A Case Report. *J Clin Med*. 2025;14(13):4432. Published 2025 Jun 22. doi:10.3390/jcm14134432 IF: 2.9
2. **Bakos A**, Libor L, Urbán S, et al. Dynamic [^{99m}Tc]Tc-mebrofenin SPECT/CT in preoperative planning of liver resection: a prospective study. *Sci Rep*. 2024;14(1):30305. Published 2024 Dec 5. doi:10.1038/s41598-024-81331-z IF: 3.9
3. **Bakos A**, Besenyi Z, Sipka G, et al. 18F-FDG-PET/CT-vel szerzett tapasztalataink az aktív nagyérvasculitisek diagnosztikájában és differenciáldiagnózisában. Prospektív vizsgálat. [18F-FDG-PET/CT in the evaluation and differential diagnosis of active large-vessel vasculitis. A prospective study]. *Orv Hetil*. 2020;161(20):829-838. Published 2020 May 1. doi:10.1556/650.2020.31710 IF: 0.54

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3. **Bakos A**, Besenyi, Z, Urbán Sz, Farkas I, Sipka G, Hemelein R, Kovács L, Pávics L 18F-FDG-PET/CT szerepe a nagyérvasculitisek diagnosztikájában MAGYAR RADIOLÓGIA 92 : 1 pp. 57-58. , 2 p. (2018)
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